PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A01N 43/90, C09B 47/04
C07D 487/22, C08K 5/00

A1

(11) International Publication Number: WO 93/00815

(43) International Publication Date: 21 January 1993 (21.01.93)

(21) International Application Number: PCT/GB92/01191

(22) International Filing Date: 1 July 1992 (01.07.92)

(30) Priority data:
9114290.1 2 July 1991 (02.07.91) GB

(71) Applicant *(for all designated States except US)*: COURTAULDS PLC [GB/GB]; 50 George Street, London W1A 2BB (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : BONNETT, Raymond [GB/GB]; Elmbank, 19 Station Road, Epping, Essex CM16 4HG (GB). BUCKLEY, Dennis, Graham [GB/GB]; 26 Somerset Road, Tunbridge Wells, Kent TN4 9PR (GB). GALIA, Aslam, Buda, Bachu [GB/GB]; 134 Gregory Avenue, Weoley Castle, Birmingham B29 5DU (GB). SAVILLE, Brian [GB/GB]; "The Chapel", Milton Lilbourne, Wiltshire SN9 5LF (GB).

(74) Agent: HALE, Stephen, Geoffrey; J.Y. & G.W. Johnson, Furnival House, 14-18 High Holborn, London WC1V 6DE (GB).

(81) Designated States: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).

Published
With international search report.

(54) Title: POLYMER COMPOSITIONS

(57) Abstract

Compositions of a polymer and a photosensitiser capable of catalysing the formation of singlet oxygen from triplet oxygen under the influence of visible light have photobactericidal properties and autosterile character on exposure to visible light. A method of sterilising a surface consists in exposing a surface containing such a photosensitiser to visible light. The composition may contain 0.1-1.0 % by weight of the photosensitiser. The photosensitiser may for example be a porphyrin or phthalocyanine, preferably in the unmetallated form. Salts of the meso-tetra(N-octyl-4-pyridinium)porphyrin tetracation have cytotoxic and photocytotoxic properties.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		
ES	Spain				

POLYMER COMPOSITIONS

Technical Field

This invention relates to polymers, polymer compositions and articles which incorporate photosensitisers, to their manufacture, to their use in applications where light-induced sterility is desired over a period of time, to methods of sterilising a surface, and to cytotoxic agents.

Background Art

Known methods of sterilisation include treatment with cytotoxic substances, for example in solution or as a gas, heat treatment, for example autoclaving, and exposure to high energy radiation, for example ultraviolet light or gamma rays. The sterility induced by such methods of treatment is not permanent, and repeated treatments may be needed to restore sterility during and after use. There would be considerable interest in materials which were autosterile, that is to say materials which had at least some inherent bactericidal and sterilising ability. It is an object of the present invention to provide such materials and articles made from them.

Oxygen is a triplet molecule in its ground state. Singlet oxygen is much more reactive than triplet oxygen and can react with biomolecules such as unsaturated lipids, cholesterol and the indole moiety of tryptophan in proteins. It is toxic to living tissue, including microorganisms.

Compounds are known which absorb electromagnetic radiation, for example visible light, to generate an excited singlet stat , which decays by the process known as intersystem crossing to yi ld a triplet stat . Such comp unds may b called photosensitisers. Some such triplet state molecul s can then react with tripl t oxygen, for

example oxygen of the air, to generate singlet oxyg n. This reaction may proceed with re-formation of the singlet state photosensitiser molecule, in which case the overall process is catalytic.

The combination of certain photosensitisers, oxygen and light has been shown to be toxic to living tissue. Such photosensitisers may be described as photocytotoxic, in that the combination is more toxic than the combination of photosensitiser and oxygen in the absence of light. This toxic effect has been called the photodynamic effect and is believed to be the consequence of the formation of singlet oxygen in such combinations. Other mechanisms, such as the formation of oxygen-containing free radicals, for example the hydroxyl radical and superoxide anion, may also be involved.

T.A. Dahl, W.R. Midden and P.E. Hartman, Photochem. Photobiol., Volume 46 (1987), page 345, describe the irradiation of a deposit of Rose Bengal to kill bacteria at a distance of 0.65 mm.

Japanese Published Unexamined Patent Publication 63-296875 describes the preparation of membranes for preserving the freshness of food. A composition which comprises a metallic phthalocyanine polycarboxylic acid and a binder resin is spray-dried onto a base film, for example polyester film. The effect appears to rely on some inherent property of the membrane rather than on any photodynamic effect, as the particular phthalocyanine suggested does not appear to be a photosensitiser.

W. Lautsch and co-workers, in Journal of Polymer Scienc , Volume 8 (1952), pages 191-213 and Volume 17 (1955), pages 479-510, describe the synthesis of high polymers carrying active groups of the chlorophyll and haemin seri s and the properties of thes polymers as nzym models of oxidase and cytochr me characteristics.

M. Kamachi and co-workers, in Journal of Polymer Science, Polymer Letters Edition, Volume 21 (1983), pages 693-698, and in Macromolecules, Volume 20 (1987), pages 2665-2669, describe the addition polymerisation of 5-(4-acryloyloxyphenyl)-10,15, 20-triphenylporphyrin.

H. Kamogawa, in Journal of Polymer Science, Polymer Letters Edition, Volume 10 (1972), pages 711-713 and in Journal of Polymer Science, Polymer Chemistry Edition, Volume 12 (1974), pages 2317-2325, describes the synthesis of polymers from polymerisable chlorophyll, porphyrin and metalloporphyrin monomers and the use of these polymers as catalysts in photoreduction reactions.

Disclosure of Invention

According to one aspect of the invention, a polymer composition is characterised in that it comprises (1) a polymer and (2) a photosensitiser which is capable of catalysing the formation of singlet oxygen from triplet oxygen under the influence of visible light and in that it exhibits bactericidal activity when the composition comprising the photosensitiser is exposed to visible light. Such compositions have autosterile character, being toxic to microorganisms when exposed to light in the absence of any other external stimulus, and are photocytotoxic. The compositions of the invention exhibit photobactericidal activity on exposure to visible light, photobactericidal activity being bactericidal activity induced by exposure to electromagnetic radiation. In the context of the present invention, bactericidal activity includes bacteriostatic activity wherein bacterial growth is inhibited.

According to another aspect of the invention, an article is characterised in that at least the surface of the article comprises a polymer composition which includes a photosensitiser capable of catalysing the formation of singlet oxygen from triplet oxygen under the influence of

visible light and in that the surface exhibits photobactericidal activity when the composition c mprising the photosensitiser is exposed to visible light. The surface may be a coated or formed surface.

According to a further aspect of the invention, a method for sterilising a surface is characterised in that the surface contains or consists of a polymer composition which includes a photosensitiser capable of catalysing the formation of singlet oxygen from triplet oxygen under the influence of visible light and in that the surface is exposed to visible light. The surface preferably consists of a polymer composition which includes the photosensitiser. The surface may be a coated or formed surface. The method for sterilising a surface according to the invention reduces the standing concentration of bacteria on the surface.

According to a further aspect of the invention, the salts of the meso-tetra(N-R-4-pyridinium)porphyrin tetracation wherein R represents a 1-hexyl or 1-octyl group are claimed as novel compounds.

The photosensitisers used in the invention are preferably stable molecules which resist attack by singlet oxygen. Such photosensitisers provide a catalytic and long-lived effect. Preferred examples of such molecules are provided by the porphyrins and the phthalocyanines. A wide range of synthetic and naturally-occurring porphyrins and of synthetic phthalocyanines has been prepared and is available, as described for example in Comprehensive Heterocyclic Chemistry, ed. A.R. Katritsky and C.W. Rees, Pergamon Press (1984), Volume 4, page 377. Other examples are provided by hydroporphyrins, naphthalocyanines, and photosensitiser dyes, for example xanthene dyes such as Rose Bengal, and azine dyes such as Methylene Blue.

Porphyrins and phthalocyanines may be us d in the invention in the metallated or unmetallated form. Preferred

m tallat d compounds contain a metal ion which does n t have a partially filled d-shell, for example aluminium and zinc. Metallated compounds containing a metal ion which has a partially filled d-shell, for example transition metals such as copper and iron, are generally not suitable for use in the invention. When such metallated compounds are exposed to light they are believed to form a triplet state which is very short-lived and therefore is not active in catalysing the formation of singlet oxygen. Such metallated compounds have been used as colorants, for example in the form of pigments, for which purpose stability and inertness to light are desirable properties.

One preferred class of photosensitiser for use in the invention consists of the salts of the meso-tetra(N-alkyl-4-pyridinium)porphyrin tetracation. The nature of the counteranion is in general not critical and may suitably be chosen for ease of synthesis and handling or for compatibility with a polymer. An example of a counteranion is tosylate (4-methylbenzenesulphonate). The alkyl group is preferably a C_1 to C_8 alkyl group, for example methyl, ethyl, butyl or octyl, more preferably a C_1 to C_4 alkyl group, most preferably a 1-butyl group. The alkyl group may be branched or unbranched. The alkyl groups may be the same or different. Other substituents may be used in place of one or more of the alkyl groups, for example aryl, aralkyl and substituted alkyl and aryl groups. Polymer compositions containing meso-tetra(N-R-4-pyridinium)porphyrin tetratosylate wherein R represents a 1-decyl or 1-dodecyl group were surprisingly found not to be useful in the invention, and articles made of these compositions did not exhibit photobactericidal behaviour. These compositions and articles were therefore not according to the invention. Polymer compositions containing meso-tetra(N-R-4-pyridinium)porphyrin tetratosylate wherein R represents a 1-hexyl or 1- ctyl group were surprisingly found to hav cytotoxic activity in both the absence and presence of light. Th 1-octyl compound was found to be more active in

the presence than in the absence of light, and therefore exhibits both photocytotoxic and cytotoxic character. The 1-hexyl and 1-octyl compounds are therefore useful as bactericidal agents in both the absence and presence of light.

The photosensitisers used in the invention preferably absorb and are activated by visible light (wavelength 400-750 nm) at every-day intensities. They are therefore coloured compounds. Use of such photosensitisers has the advantage that daylight or normal artificial light can be used to induce bactericidal activity and consequent sterilisation. It has the further advantage that high-energy radiation such as ultraviolet light or gamma rays is not required to induce sterilisation. Such high-energy radiation may have harmful biological effects and may alter the properties of the material to be sterilised.

The proportion of photosensitiser in a polymer composition or article according to the invention may be varied over a wide range, depending on the activity of the photosensitiser and on the desired level of photobactericidal and sterilising activity. It may be as low as 0.1 or 0.01% by weight or even lower, so providing mildly photobactericidal compositions and articles. Use of a low level of photosensitiser has the advantage that the degree of coloration of the composition or article due to the presence of the photosensitiser is low. Higher proportions of up to 1 or 10% by weight or more provide more strongly photobactericidal compositions. Proportions in the range 0.1 to 1% by weight may be preferred.

A variety of polymers can be used in the invention. The polymer is preferably chosen to resist attack by singlet oxygen. The invention is not limited by the class of polymer. Examples of suitable polymers include, but are not limited to, regenerated cellulose, for example viscose rayon; cellulose esters, for example cellulose acetate; and

addition polymers, for example polyolefins and olefinic polymers.

Porphyrins, phthalocyanines and other photosensitisers having a variety of different chemical functionalities and characteristics are known, and suitable functionalities and characteristics can be selected for reactivity with or compatibility towards a particular polymer.

Polymer compositions which include a photosensitiser may be prepared in a variety of ways. In one embodiment of the invention, a polymer is dyed using a solution of the photosensitiser in a suitable solvent. The polymer may for example be in the form of a fibre or film. The polymer may be a natural polymer, for example cotton, or an artificial polymer, for example viscose rayon. In another embodiment of the invention, the photosensitiser is included in a polymer melt or dope which is then formed by casting, moulding or extrusion to produce a solid article. The photosensitiser may be dissolved or dispersed in the melt or dope. Such methods of casting, moulding and extrusion are well known in the field of polymer manufacture. In a further embodiment of the invention, the photosensitiser is a chemical entity bonded to a polymer, so being a photosensitive polymer. Such polymers may be made by the inclusion of one or more suitable photosensitive monomers containing a photosensitiser group in a polymerisation reaction, for example an addition polymerisation, so as to incorporate the photosensitiser in the polymer. Such polymers may alternatively be made by reacting a photosensitiser or precursor thereof with a polymer, so grafting photosensitiser molecules onto a polymer, for example in the form of dependent groups. The polymer composition of the invention may be a coating composition.

Photobactericidal articles according to the invention can be manufactured in a variety of physical forms. For example, a polymer composition which includes a

photosensitiser can be utilised in the form of coatings, films, fibres or pellets, optionally incorporat d into more complex articles. For example, fibres may be converted into woven, knitted or non-woven textile articles. It is a feature of the photobactericidal articles of the invention that they provide a photobactericidal surface.

The photobactericidal articles of the invention may take the form of textile articles, for example cleaning cloths, wipes, surgeons' gowns, bedlinen, wound dressings and bandages. They may alternatively take the form of self-supporting films, for example for use in food packaging or wound dressings. Wound dressings may be sterilised by exposure to light before application and then covered up, once they have been placed over a wound, to restrict or eliminate exposure to light in order to stop the formation of singlet oxygen, which may be harmful and consequently undesirable in a wound environment. The compositions and articles of the invention are useful in medical and clinical auxiliaries for domestic and hospital use, for example tubing, bags and mats used in dialysis procedures, for example kidney dialysis. The articles may take the form of polymer laminates with a photobactericidal surface for use in hygienic applications, in which one or more layers of the laminate contains or consists of a polymer composition which includes a photosensitiser. Preferably at least one surface layer of the laminate contains or consists of a polymer composition which includes a photosensitiser. The articles may take the form of a substrate having a photobactericidal coated or painted surface. The substrate can for example be wood, metal, glass or plastic, but is not limited thereto. A particular aspect of the invention is to provide a surface on an architectural or underwater article which inhibits the growth and adhesion of organisms such as algae and bacteria when exposed to light. The articles may take the form of polymer beads. Such beads are of use in water treatment plants in which a bed or column of such beads exposed to sunlight is us d to inhibit the growth of and to kill

organisms such as bacteria and algae.

The method for sterilising a surface according to the invention has a number of advantages over known methods of sterilising surfaces. The rate of production of the sterilising batericide, believed to be singlet oxygen, can be controlled by varying the intensity of irradiation by visible light, either generally or in selected areas. This control is in addition to that provided by the activity and concentration of the photosensitiser. It is an advantage of the invention that no special equipment or treatment is required to induce bactericidal activity. It is a further advantage of the invention that the bactericidal effect continues during exposure to light and diminishes only little with the passage of time. It is an advantage of the invention that repeated treatments with a sterilising agent are not required.

Brief Description of Drawings

The invention is illustrated by the following Examples with reference to the accompanying Figures I-III, in which:

Figure I shows the structure of meso-tetra(N-methyl-4-pyridinium)porphyrin tetratosylate;

Figure II shows the structure of tetra-t-butylphthalocyanine; and

Figure III shows the structure of protoporphyrin dimethyl ester.

Modes for Carrying out the Invention

All parts and percentages in the Exampl s which follow are by weight unless otherwise specified.

Example 1

1. Preparation of alkyl tosylates

1.1. Preparation of ethyl tosylate

Ethanol (2.36 g, 50 mmol) was mixed with tosyl chloride (5.01 g, 26 mmol) and cooled to 0°C in an ice-salt bath. Pyridine (4.89 g) was added dropwise to the mixture over 2 hours. The solution was then acidified with dilute HCl (50 ml) and extracted with ether (3 x 50 ml). The ether extract was dried (K_2CO_3), filtered and evaporated to yield ethyl tosylate (3.51g, 66%) as a white solid (m.pt. 32-33°C).

1.2. Preparation of butyl tosylate

Butan-1-ol (3.70 g, 50 mmol) was reacted with tosyl chloride (10.50 g, 50 mmol) and pyridine (8.51 g) in the manner described for ethyl tosylate. The product was distilled (162°C/0.5 mm Hg) to yield butyl tosylate (10.61 g, 86%).

1.3. Preparation of octyl tosylate

Octan-1-ol (8.01 g, 100 mmol) was dissolved in pyridine (20.02 g) and cooled to below 0°C. Tosyl chloride (12.04 g, 60 mmol) was added to the mixture over 2 hours while maintaining the temperature below 0°C, and the mixture stirred for a further hour. The product was extracted as described for ethyl tosylate and distilled (155°C/0.1 mm Hg) to yield octyl tosylate (9.04 g, 77%).

1.4. Preparati n of dodecyl tosylate

Dodecan-1-ol (8.07 g, 40 mmol), pyridine (20.05 g) and tosyl chlorid (6.01 g, 30 mmol) were reacted as described

for octyl tosylate. Dodecyl tosylate (7.89 g, 76%) was obtained in the form of a white solid (m.pt. 27-28°C).

2. Preparation of meso-tetra(4-pyridyl)porphyrin (TPyP)

A mixture of pyrrole (3.32 g, 48 mmol), pyridine-4-carboxaldehyde (5.14 g, 48 mmol) and propionic acid (200 ml) was heated under reflux for 45 minutes. Solvent was removed under vacuum and the residue washed with DMF (200 ml) to remove tarry byproducts and leave a purple crystalline material. The residue was further washed with DMF (2 x 20 ml and 4 x 10 ml) and ether (3 x 20 ml), allowed to dry at room temperature in the dark, and recrystallised from chloroform

to yield purple crystals of meso-tetra(4-pyridyl)porphyrin (TPyP) (1.46 g, 20%).

3. Preparation of meso-tetra(N-alkyl-4-pyridinium)porphyrin salts (TRPyP)

3.1. Preparation of meso-tetra(N-methyl-4-pyridinium)porphyrin tetratosylate (TMPyP)

A solution of TPyP (1 g, 1.6 mmol) and methyl tosylate (2 g, 11 mmol) in DMF (60 ml) was heated under reflux for 6 hours and cooled gradually to 0°C. The product was collected by filtration, washed with water (100 ml), and dried in a desiccator to yield TMPyP (1.96 g, 90%). The structure of TMPyP is shown in Figure I.

3.2. Preparation of meso-tetra(N-ethyl-4-pyridinium)porphyrin tetratosylate (TEtPyP)

A mixture of ethyl tosylate (1.21 g, 6.0 mmol), TPyP (0.175 g, 0.28 mmol) and DMF (10 ml) was heated at 85°C f r 16 hours and cooled slowly to 0°C. The product was collected by filtration, wash d with acetone, dried, and recrystallised from methanol/acetone to yield TEtPyP (60 mg, 26%) in the f rm of

a fine purple powder.

3.3. Preparation of meso-tetra(N-butyl-4-pyridinium)porphyrin tetratosylate (TBuPyP)

Butyl tosylate (1.01 g, 4.46 mmol), TPyP (209 mg, 0.30 mmol) and DMF (3 ml) were heated at 140°C for 4 hours. The mixture was cooled to room temperature and stored overnight at -5°C. Solid product was collected by filtration, washed with acetone and dried to yield TBuPyP (473 mg, 96%). A small sample was purified by recrystallisation from methanol/acetone to yield a purple powder.

3.4. Preparation of meso-tetra(N-octyl-4-pyridinium)porphyrin tetratosylate (TOcPyP)

Octyl tosylate (1.03 g, 3.61 mmol), TPyP (203 mg, 0.33 mmol) and DMF (5 ml) were heated at 140°C for 5 hours. The mixture was cooled to room temperature and stored overnight at -5°C. Solid product was collected by lengthy filtration, washed with acetone, recrystallised from methanol/acetone, and dried to yield TOcPyP (350 mg, 62%) in the form of a brown-purple powder.

3.5. Preparation of meso-tetra(N-dodecyl-4-pyridinium) porphyrin tetratosylate (TDoPyP)

Dodecyl tosylate (0.51 g, 3.6 mmol), TPyP (197 mg, 0.32 mmol) and DMF (3 ml) were heated at 140°C for 9 hours. Dodecyl tosylate (0.52 g, 3.6 mmol) and DMF (2 ml) were added to the mixture and heating continued for a further 15 hours. The mixture was cooled to room temperature and stored overnight at -5°C. Solid product was collected by lengthy filtration, washed with acetone, recrystallised from methanol/aceton , and dried to yield TDoPyP (490 mg, 76%) in the form of a dark brown-purple powder.

The tetratosylates w re characterised by proton NMR. All the tetratosylates were very soluble in methanol. TMPyP and TEtPyP were very soluble in water, TBuPyP was soluble, and TOcPyP and TDoPyP were slightly soluble.

4. Dyeing of regenerated cellulose film with TRPyP

Squares (2 cm x 2 cm) of regenerated cellulose film (50 micron thick) were placed in water or methanol (10 ml) containing TRPyP (1 mg) for 12 hours at room temperature or for 5 minutes at reflux. The films were then washed with solvent and dried between filter papers. Qualitative results are shown in the following Table, where '+' represents successful and '-' unsuccessful dyeing:

	12hrs / room temp.		5 mins / reflux	
	H_2O	MeOH	H_2O	MeOH
TMPyP	+	+	+	+
TEtPyP	+	+	+	+
TBuPyP	+	-	+	-
TOcPyP	+	-	+	-
TDoPyP	+	-	+	-

Dyeing with aqueous solutions of TOcPyP and TDoPyP was not completely satisfactory because of the low solubility of these two compounds in water. Satisfactory dyeing was obtained using solutions of these compounds in a mixture of 6 parts methanol and 5 parts water by volume at 40°C.

Example 2

A regenerated viscose film 25 micron thick was refluxed in 10^{-3}M TMPyP in solution in methanol or water. The film was dyed to a yellow-brown colour, and showed absorption maxima at 520, 556, 606 and 641 nm, the absorbance at 520 nm being 0.20.

Sterile agar plates were prepared and a sample of sterile undy d or dyed film 3 cm square placed in contact with the top

surface of the agar on each plate. A standard amount (20 µl) of a bacterial culture was placed on top of the film, and th plates incubated either in the dark or illuminated by a 100 watt electric lamp. Plates inoculated with Escherichia coli were incubated at 37°C for 48 hours, and those with Bacillus subtilis and Micrococcus luteus at 27°C for 24 hours. Bacterial growth was observed on all squares of film incubated in the dark and on the undyed square of film incubated in the light. No growth of E. coli or B. subtilis and slight growth of M. luteus was observed on dyed squares of film incubated in the light.

Example 3

1. Dyeing of regenerated cellulose film with TRPyP

TMPyP (50.3 mg) was dissolved in 6:5 methanol/water (110 ml) at 40°C to give a 1.39 mM solution. Four squares (4 cm x 4 cm) of regenerated cellulose film (50 micron thick) were successively dyed in the solution, being immersed for 1, 3, 5 and 10 minutes respectively. The strips were thoroughly washed in 6:5 methanol/water at 40°C and dried. The same procedure was used to dye film using the other porphyrins: TEtPyP (32.8 mg, 1.38 mM), TBuPyP (50.5 mg, 1.22 mM), TOcPyP (50.0 mg, 1.07 mM) and TDoPyP (50.1 mg, 0.91 mM). The concentration of porphyrin in the dyed films was measured by visible spectroscopy at the 519 nm absorption band, and the films were found to contain the following amounts of porphyrin (in mg):

	Immersion time min.			
	1	3	5	10
TMPyP	0.25	0.34	0.58	1.29
TEtPyP	0.15	0.50	1.04	1.21
TBuPyP	0.09	0.40	0.48	1.26
TOcPyP	0.25	0.34	0.59	0.99
TDoPyP	0.22	0.55	0.58	0.84

2. Cytotoxicity of regenerated cellulose film dyed with TRPyP

Five different squares of dyed film and a squar of undy d film as a control (all 2 cm x 2 cm) were placed on th surface of an agar plate. Each square was inoculated with a culture of Staphylococcus aureus. The plates were incubated for 24 hours at 37°C either in the light (8 watt fluorescent tube at 30 cm) or in the dark. Bacterial growth was assessed visually and is recorded in the following Table:

	Culture in light				Culture in dark			
	Immersion time min.				Immersion Time min.			
	1	3	5	10	1	3	5	10
TMPyP	2	1	1	1	5	5	5	5
TEtPyP	3	1	1	1	5	5	5	5
TBuPyP	1	1	1	1	5	5	5	5
TOcPyP	1	1	1	1	2	1	1	1
TDoPyP	5	5	5	5	5	5	5	5

In the above Table, 1 represents no bacterial growth; 2 fewer than five colonies; 3 patchy bacterial growth; 4 medium growth; and 5 maximum bacterial growth, corresponding to the control.

It will be observed that the films impregnated with TMPyP, TEtPyP and TBuPyP were photocytotoxic in that they exhibited bactericidal behaviour in the light but not in the dark. The films impregnated with TOcPyP were surprisingly both cytotoxic and photocytotoxic in that they exhibited autosterile behaviour in both the dark and the light. A minimum concentration of 0.02 $mgcm^{-2}$ TOcPyP in the film was required to kill all bacteria in the dark. The films impregnated with TDoPyP exhibited neither cytotoxic nor photocytotoxic properties and were therefore not according to the invention.

Example 4

A solution containing cellulose diacetat (71.8 g), diethyl phthalate (7.1 g), tetra-t-butylphthalocyanin (10 mg), wat r (7.0 g) and acetone (360 g) was prepared. This was spread

on glass plates and the solvent allowed to evaporate, to yield a pale blue film having absorption maxima at 615, 634, 664 and 698 nm. The structure of tetra-t-butylphthalocyanine is shown in Figure II.

Example 5

Protoporphyrin dimethyl ester (PDME, 10 parts) and methyl methacrylate (17 parts) were polymerised in solution in dimethyl formamide, using the thermal degradation of azobisisobutyronitrile (AIBN, 5.4 parts) to initiate the reaction. A light brown polymer (23 parts) was recovered. Films were prepared by spreading a solution of the polymer in chloroform on glass plates and allowing the solvent to evaporate. These films showed absorption maxima at 503, 536, 570, 624 and 661 nm. The structure of protoporphyrin dimethyl ester is shown in Figure III.

Example 6

Example 5 was repeated, except that the weight ratio of methyl methacrylate to PDME was 50:1. A dark brown polymer was obtained.

Example 7

Example 5 was repeated, except that the weight ratio of methyl methacrylate to PDME was 400:1. A light brown polymer was obtained.

Example 8

Example 5 was repeated, except that divinylbenzene and PDME in a weight ratio of 100:1 were used. A dark brown polymer was obtained.

Example 9

Example 5 was repeated, except that styrene and PDME in

a weight ratio of 50:1 were used. A light brown polymer was obtained.

Example 10

Tetra-5,10,15,20-(4-hydroxyphenyl)porphyrin was treated with excess acrylic anhydride and potassium carbonate in solution in dimethyl formamide to prepare the tetraacrylate. This was heated under reduced pressure to produce a purple polymer.

Example 11

5-(4-Hydroxyphenyl)-10,15,20-triphenylporphyrin (MHTTP) was prepared from pyrrole, benzaldehyde and 4-hydroxybenzaldehyde as described by Little in Journal of Heterocyclic Chemistry, Volume 12 (1975), page 343. The crude mixture was purified by chromatography on silica gel using chloroform as eluant to yield pure MHTTP as a purple solid (4.7% based on pyrrole).

MHTTP (18.4 mg, 0.029 mmol) and anhydrous potassium carbonate (60 mg) were stirred in DMF (2.5 ml) and excess methacryloyl chloride (50 µl) was added. The mixture was stirred for 26 hours at room temperature in the dark, further methacryloyl chloride (15 µl) was added, and the mixture was stirred for a further hour. After filtration, solvent was removed by evaporation to yield a purple powder which was recrystallised from chloroform/methanol to provide purple crystals of pure 5-(4-methacryloyloxyphenyl)-10,15,20-triphenylporphyrin (MAOTTP) (78% yield).

MAOTTP (2 mg) and AIBN (1 mg) were dissolved in DMF (1 ml), and the solution heated to 85°C with stirring. A further portion of AIBN (0.5 mg) was added after 96 hours, and heating continued for a further 24 hours, at which point thin-layer chromatography showed that all MAOTTP had been consumed. The reaction mixture was filtered and solvent removed to yield poly-

MAOTTP (1.2 mg, 60%) as a dark purple powder. The polymeric product exhibited absorption bands at 419, 515, 550, 591 and 645 nm.

MAOTTP (1.9 mg) and AIBN (0.5 mg) were dissolved in DMF (3 ml). To this solution methyl methacrylate (15 µl) was added in one portion. The stirred mixture was heated at 85°C for a total of 144 hours, extra portions of AIBN being added after 96 hours (0.5 mg) and 120 hours (1.5 mg). Solvent was removed under reduced pressure to yield a light brown powder which was precipitated from chloroform-methanol (10.8 mg, 78%). The polymeric product exhibited absorption bands at 418, 516, 544, 589 and 649 nm. Analysis by gel permeation chromatography showed it to have Mw 55000 and Mn 31000 against a polystyrene standard. The copolymer could be cast as a film from solution in chloroform.

MAOTTP (6 mg) and AIBN (2.5 mg) were dissolved in DMF (3 ml). Styrene (50 µl) was added in one portion and the mixture heated at 85°C for 48 hours. Filtration and removal of solvent gave the polymeric product in the form of a light brownish-purple powder (34.2 mg, 72%).

Example 12

Regenerated cellulose film was dyed with a 2×10^{-4}M solution of TMPyP in water for 30 minutes at 50°C. The dyed film was irradiated by a xenon arc lamp for times up to 50 hours and its visible spectrum recorded. 50 hours' exposure corresponds to 20 days' continuous sunlight. The colour of the film changed gradually during irradiation from pinkish brown to greenish brown. The visible spectrum showed the gradual disappearance of the absorption peak at 641 nm and the gradual appearance of an absorption peak at 663 nm.

Electron spin resonance studies were carried out on undyed and dyed film. There was no evidence of unpaired electrons in the undyed film. The results on the dyed film showed evid nc

for the presence of a free radical species whose concentration increased during irradiation by the xenon arc lamp.

The tensile properties of the dyed film were measured before and after irradiation, with the following results:

Time	Breaking Load	Extensibility
hr	N	%
0	47.0	21
4	46.7	23
8	40.5	21
18.5	43.7	20
50.5	36.7	16

The cytotoxicity towards S. aureus of non-irradiated film and of film which had been irradiated for 50 hours was tested in the manner of Example 3, with the following results:

	Culture in light	Culture in dark
Undyed	5	5
Dyed	1	5
Dyed irradiated	1	5

Example 13

Regenerated cellulose film was dyed for various times using a solution of TMPyP (10 mg) in water (150 ml) (4.9×10^{-5}M) at 43°C. The absorbance of the samples of film at 519 nm was measured and used to estimate the concentration of TMPyP in the film. The results were as follows:

Immersion Time	Absorbance	Concentration mgm^{-2}
10 sec	0.035	26
30 sec	0.058	45
1 min	0.067	50
5 min	0.17	130
10 min	0.23	177
15 min	0.26	200

The cytotoxicity of the dyed samples of film towards S.aureus, E. coli, Proteus vulgaris and Pseudomonas aeruginosa was tested in the manner of Example 3. Bacterial growth was observed on all plates cultured in the dark. The results on the plates cultured in the light were as follows:

Absorbance	S. aureus	E. coli	P. vulgaris	P. aeruginosa
0.035	4	5	5	5
0.058	3	5	5	5
0.067	3	4	4	5
0.17	1	1	1	5
0.23	1	1	1	5
0.26	1	1	1	5

Example 14

Regenerated cellulose film was dyed with meso-tetra(4-trimethylammoniophenyl)porphyrin available from Aldrich Chemical Company Limited and tested for cytotoxic activity in the manner of Example 3. No bacterial growth was observed on plates incubated in the light (rating 1). Bacterial growth was observed on plates incubated in the dark (rating 5).

Example 15

1. Preparation of meso-tetra(N-hexyl-4-pyridinium)porphyrin tetratosylate (THePyP)

Hexyl tosylate (1.0 g, 3.9 mmol), TPyP (30 mg, 0.048 mmol) and DMF (3 ml) were heated at 140°C for 20 hours. The solution was allowed to cool to room temperature and stored overnight at -5°C. The solid product was collected by filtration, washed with acetone, and recrystallised from methanol/acetone to yield THePyP (31.2 mg, 39%) as a purple powder.

2. Preparation of mes -tetra(N-decyl-4-pyridinium)porphyrin tetratosylate (TDePyP)

Decyl tosylate (1.0 g, 3.2 mmol), TPyP (25 mg, 0.04 mmol) and DMF (3 ml) were heated at 140°C for 20 hours. The solution was allowed to cool to room temperature and stored overnight at -5°. The solid product was collected by lengthy filtration, washed with acetone, and recrystallised from methanol/acetone to yield TDePyP (27.8 mg, 37%) as a dark brown powder.

3. Assessment of cytotoxicity

Regenerated cellulose film was dyed with either THePyP or TDePyP and tested for cytotoxic activity in the manner of Example 3. No bacterial growth was observed on squares of film inoculated with THePyP incubated in either the light or the dark. Bacterial growth was observed on squares of film inoculated with TDePyP incubated in either the light or the dark.

Example 16

Example 3 was repeated, except that TBuPyP, THePyP and TOcPyP were tested. After conclusion of the incubation in light, the agar plate carrying the films was incubated in the dark for a further 72 hours. No bacterial growth was observed on any of the dyed films. This demonstrated that at least the combination of film dyed with TBuPyP and light exhibited bactericidal behaviour.

CLAIMS

1. A polymer composition, characterised in that it comprises (1) a polymer and (2) a photosensitiser which is capable of catalysing the formation of singlet oxygen from triplet oxygen under the influence of visible light, and in that it exhibits bactericidal activity when the composition comprising the photosensitiser is exposed to visible light.

2. A composition according to claim 1, characterised in that the photosensitiser is a porphyrin or a phthalocyanine.

3. A composition according to claim 2, characterised in that the photosensitiser is in the unmetallated form.

4. A composition according to claim 3, characterised in that the photosensitiser is a salt of the meso-tetra(N-alkyl-4-pyridinium)porphyrin tetracation.

5. A composition according to claim 4, characterised in that the alkyl group is a C_1 to C_8 alkyl group.

6. A composition according to any preceding claim, characterised in that the composition contains 0.1 to 10% by weight of the photosensitiser.

7. A composition according to any preceding claim, characterised in that it is made by dyeing the polymer with a solution of the photosensitiser.

8. A composition according to any of claims 1 to 6, characterised in that the photosensitiser is included in a melt or dope of the polymer which is then formed into a solid article.

9. An article, characterised in that at least th surfac of the article comprises a polymer composition which includes

a photosensitiser capable of catalysing the formation of singlet oxygen from triplet oxygen under the influence of visible light and in that the surface exhibits photobactericidal activity when the composition comprising the photosensitiser is exposed to visible light.

10. An article according to claim 9, characterised in that the polymer composition contains or consists of a photosensitive polymer.

11. An article according to claim 10, characterised in that the photosensitive polymer is an addition polymer which incorporates a photosensitive monomer.

12. An article according to any of claims 9 to 11, characterised in that the autosterile surface is formed by coating the article with the polymer composition.

13. An article according to any of claims 9 to 12, characterised in that the article is a fibre.

14. An article according to any of claims 9 to 12, characterised in that the article is a textile article.

15. A method for sterilising a surface, characterised in that the surface contains or consists of a polymer composition which includes a photosensitiser capable of catalysing the formation of singlet oxygen from triplet oxygen under the influence of visible light, and in that the surface is exposed to visible light.

16. The salts of the meso-tetra(N-R-4-pyridinium) porphyrin tetracation wherein R represents a 1-hexyl or 1-octyl group.

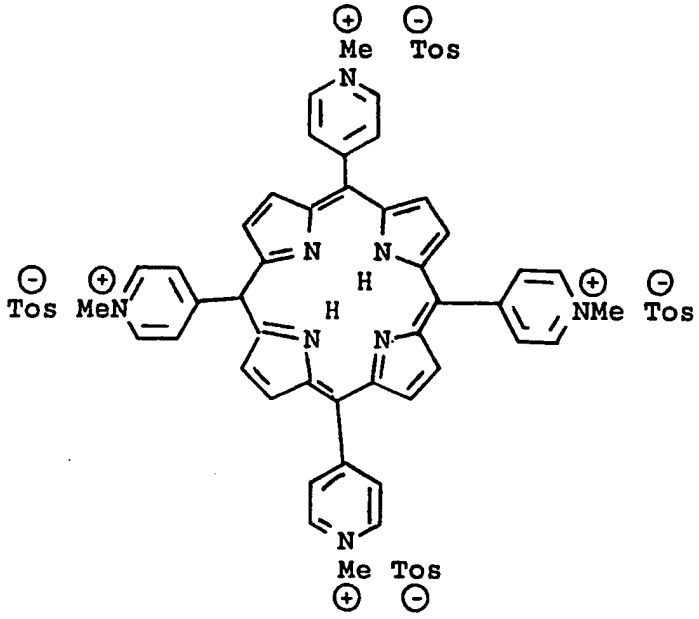

FIG. 1

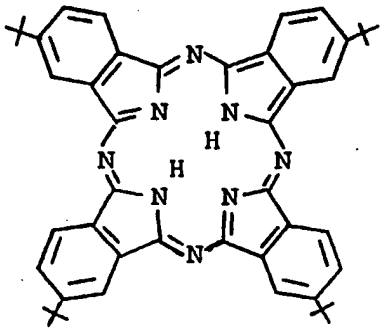

FIG. 2

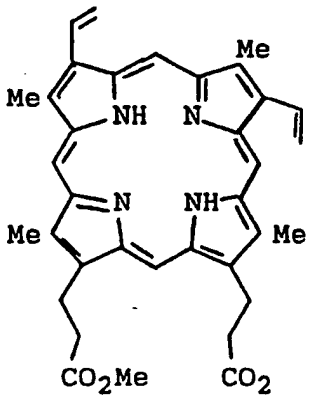

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/01191

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A01N43/90; C09B47/04; C07D487/22; C08K5/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A01N ; C09B ; C07D ; C08L C08K ; C08B

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	DATABASE WPIL Week 8122, Derwent Publications Ltd., London, GB; AN 81-39258D & JP,A,56 039 004 (ASAHI DOW KK) 14 April 1981 see abstract ---	1,2,7,9, 15
X	CHEMICAL ABSTRACTS, vol. 105, no. 19, 10 November 1986, Columbus, Ohio, US; abstract no. 172135a, see abstract & ARM. KHIM.\ZH. vol. 38, no. 6, 1985, pages 391 - 6 MADAKYAN V.N., ET AL 'N,N',N'',N'''-Tetrakis(1-alkylpyridinium-4-yl)mesoporphines and their biol. activity' --- -/--	16

* Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23 OCTOBER 1992	29. 10. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT FFICE	De Los Arcos E.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	J. PHYS. CHEM. vol. 94, no. 5, 1990, pages 2181 - 2187 KOJI KANO ET AL. 'Cationic porphyrins in water. 1H NMR and fluorescence studies on dimer and molecular complex formation' see Experimental section	16
A	DATABASE WPI Week 7512, Derwent Publications Ltd., London, GB; AN 75-20132W & JP,A,49 131 291 (BUREAU IND. TECHNOLOGY) 16 December 1974 see abstract	1,10
A	DATABASE WPIL Week 8903, Derwent Publications Ltd., London, GB; AN 89-018645 & JP,A,63 293 543 (AGENCY OF IND. SCI. TECH.) 30 November 1988 see abstract	1,8
A	DE,A,3 924 815 (W. WOLTERS) 31 January 1991 see claims	1-3,6
A	WO,A,9 006 955 (ALLIED-SIGNAL INC) 28 June 1990 see claims	1,11

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9201191 SA 62131

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 23/10/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A-3924815	31-01-91	None	
WO-A-9006955	28-06-90	US-A- 4983670	08-01-91
		EP-A- 0449880	09-10-91
		US-A- 4986921	22-01-91